COMMENTARY

Should sperm DNA fragmentation testing be included in the male infertility work-up?

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Abstract  A response to the editorial 'Are we ready to incorporate sperm DNA fragmentation testing into our male infertility work-up? A plea for more robust studies' by Erma Drobnis and Martin Johnson. © 2015 Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

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Introduction

I read with interest the recent article by Osman et al. (2015) in Reproductive Biomedicine Medicine Online and the accompanying editorial by Drobnis and Johnson (2015). I was hoping for a progressive approach but, alas, despite further confirmatory studies since the last systematic review by Zini and Sigman (2009), these authors seemed determined to draw the same, well-worn conclusions.

Those of us working in this field are equally keen to see more well-designed, adequately powered studies with live birth outcomes. We are also, however, too well aware of the hurdles and time required to achieve these ideals. Having attempted to secure funding for just such a study with El-Toukhy and other colleagues in centres excelling in andrology, we all know that this topic is not a priority for the Medical Research Council or the National Institute for Health Research. In the intervening time, until such data are available, is it still best practice to provide men with only inadequate semen testing knowing its limitations?

May I address a number of the specific, perceived shortcomings of sperm DNA damage testing?

Will the results change practice? Will testing add to the information already provided when taking a medical history?

Yes. Clearly, those men who have high levels of sperm DNA damage would be more likely to achieve a pregnancy with intracytoplasmic sperm injection (ICSI) than IVF because sperm with DNA damage do not seem to produce adverse affects on early ICSI outcomes. Why? This may be for a number of related reasons associated with the ability of the oocyte to repair at least some sperm DNA damage before first cleavage. First, unlike IVF, up to 30% of women having ICSI have no detectable problems. They may be fertile and their oocytes may have more capacity to repair DNA damage even if the injected sperm is of poor quality. This is supported by Meseguer et al. (2011), who found that high-quality oocytes from donors offset the negative effect of sperm DNA damage on pregnancy.

Second, in ICSI, the gametes are not subjected to prolonged culture so the sperm may have less damage than those exposed to culture media overnight, as in IVF procedures. The studies from Dumoulin et al. (2010) and Kleijkers et al. (2014) show that, for IVF babies, the birth weight and 2-year growth
markers can be markedly influenced by minor differences in culture conditions. In contrast to IVF, ICSI sperm are injected into the optimal environment of the ooplasm within a few hours of ejaculation. This may protect them from laboratory-induced damage and allow the oocyte to begin repair earlier.

Third, it is well documented that sperm from up to 40% of infertile men have high levels of reactive oxygen species (ROS) (Aitken et al., 2012), and their antioxidant content is also significantly lower than fertile men (Lewis et al., 1995). During the IVF process, oocytes can be exposed to oxidative assault overnight from up to 0.5 million sperm releasing ROS. This may well impair the oocyte's functional capacity to repair sperm DNA fragmentation (DNA-F) after fertilization and before cleavage.

There are patients with normal semen parameters and high DNA-F; does this mean all men should be tested during work-up?

Again, the answer must be yes. All men with normozoospermic semen profiles should be tested. Most clinicians agree on the limitations of conventional semen analysis. We also know that about 25% of couples are diagnosed with idiopathic infertility as a result of the limitations of current testing. Couples with unexplained infertility perform less well after IVF than couples with detectable causes of infertility, added to which a significant number of the male partners have high sperm DNA damage (Oleszczuk et al., 2013; Simon et al., 2013). Now that we have found a test that can detect molecular anomalies in sperm, why would we not use it as an adjunct to the semen analysis? This is not just 'an emotionally satisfying' test as suggested in the editorial. It is a more sensitive molecular test that looks at the paternal genome; the only sperm parameter that affects offspring health.

Is DNA testing better than any previous andrological test?

Yes. It is, however, being withheld from clinical use by excessively rigorous demands for evidence of quality. In most reviews of this nature, there is a common style. All previous inadequate andrological tests (except semen analysis) are listed and dismissed within one sentence. Then, this one test is expected to pass scrutiny at the highest level: Level 1 evidence of quality from randomized controlled trials (RCTs), before clinical use. This is in stark contrast to the approach used for ICSI in 1995, when it became a routine publically funded fertility treatment on a global scale without human trials. A second example of a new fertility technology becoming routine before passing such stringent standards is the use of time-lapse imaging. It has become a routine tool for many clinics, despite limited and conflicting evidence or improved assisted reproduction technique outcomes. Further, a review (Glujovsky et al., 2012) of the quality of RCTs between 2006 and 2011 in five leading human reproduction journals pointed out that incomplete outcome data and inadequate allocation concealment led to bias in almost 50% of them. Hence, to wait for a useful RCT in this area may not be in patient's best interest.

The current ‘gold standard’ semen analysis would not pass a Level 1 evidence of quality at any level: whether based on accuracy, reproducibility, diagnostic or prognostic usefulness. I dispute the authors’ comment on the sensitivity and specificity of a semen analysis being useful. The values they cited were for natural fertility not assisted conception, and they were based on studies conducted between 1997 and 2001. Reviews by Lefèvre et al. (2007) and the British Fertility Society andrology guidelines (Tomlinson et al., 2013) highlight the limitations of the semen analysis. This is not surprising, as only 1% of sperm reach the site of fertilisation in vivo, so to expect an analysis of an ejaculate’s gross parameters to give strong discriminatory information is also unrealistic. In its current form, a semen analysis should be considered only as a means of identifying men whose chance of achieving a natural pregnancy is reduced (Lewis et al., 2013). Knowing this, however, suggests an additional test needs to be implemented today.

Most existing studies evaluating the utility of DNA-F testing for the diagnosis of infertility suffer from multiple weaknesses as discussed recently in an American Society for Reproductive Medicine Practice Committee guideline, 2013

The American Society of Reproductive Medicine (ASRM) cited here, is, in my opinion, a negatively biased and unbalanced overview. It dismissed the findings of around 100 papers in high-impact journals over the last 30 years as ‘insufficient evidence’ and turned the evidence from the range of different sperm DNA tests measuring different aspects of DNA damage into a weakness rather than accepting this as the strength it is.

The level of precision that ASRM requires can never come from one ‘male only’ test. Fertilization is a multi-factorial process and a successful assisted reproduction technique outcome depends on many other traits of sperm quality and function, as well as the influences of the oocyte, uterine receptivity and maternal immune system competence. Finally, the authors of the ASRM document, having begun by criticizing DNA testing as measuring different aspects of DNA damage and, therefore, being non-uniform, then criticized DNA testing for being non-specific by not providing ‘an indication of specific DNA sequences that may be affected’. As no one yet knows what aspects of DNA damage at large, or of any specific sequences that are responsible for ‘male infertility’, neither of these opposing criticisms is based on sound science.

As a co-author of the European Society of Human Reproduction and Embryology guidelines (Barratt et al., 2011), I am always surprised to see them cited in opposition to sperm DNA testing. The paper was written as a broad scientific overview, as the title suggests ‘Sperm DNA: organization, protection and vulnerability: from basic science to clinical applications – a position report.’ Its coverage of clinical testing was far from exhaustive, citing only a few reviews and composing less than 10% of the paper content. We ended with a plea for further funding for fundamental male reproduction. That was 5 years ago and the plea remains unheeded.
Again, I ask, should infertile couples be disadvantaged by refusing to offer them these tests while we wait for further supportive data that may take years to fund?

It will be important to compare the value of DNA-F testing with other tests that could provide the same information, for example, a combination of semen analysis and reactive oxygen species concentration

The advantage of sperm DNA testing is that it adds to our current knowledge. No other tests provide the same information. In a study of 1633 semen samples tested for 21 parameters, only seven of them showed any correlation between sperm DNA (Cohen-Bacie et al., 2009). If sperm DNA damage correlated with a semen analysis, then DNA testing would be unnecessary. In response to the authors’ comment about a relationship with ROS, this is a difficult parameter to measure as it has a half-life of milliseconds. Much evidence already shows that most sperm DNA damage is caused by oxidative stress (reviewed by Aitken et al., 2012), so sperm DNA damage is accepted as a robust end point of irreversible damage by this process.

Beyond diagnosing male factor infertility, can we use DNA-F testing to decide which interventions will best treat our infertile couples? If so, significant cost and risk to the patient could be minimized. The ideal study would recruit men with positive and negative DNA-F tests, and randomly assign them to different treatments: expectant management versus intrauterine insemination versus IVF versus IVF–ICSI

I agree. Intrauterine insemination (IUI) is no longer recommended by the National Institute for Health and Care Excellence, but a study of couples randomly allocated to IVF or ICSI could be useful. I have proposed this type of study to clinical colleagues on many occasions. It has always been met with ethical disapproval, however. Some clinics objected as they were averse to performing ICSI on couples with normal semen as it was too invasive. Other clinics objected for the converse reason; they used ICSI rather than IVF as the more successful treatment for most patients. A further hurdle is that couples are often self-funded and, when they are fully informed of the details of such a study, they do not want to jeopardise their chances of success by choosing a treatment that they perceive as less successful.

In closing, I would highlight two new publications from Doug Carrell’s group (Simon et al., 2014a, 2014b). These add further support to the benefits of sperm DNA testing and show the strong affect of the paternal genome on fertilization and early embryo quality giving us new possibilities to incorporate male testing with time-lapse imaging.

We have two choices. We can allow this inertia to continue and our traditional tests to prevail, but that may prevent an improvement in our success rates in the diagnosis and treatment of male infertility. Alternatively, we can embrace innovation in male fertility testing and see if success rates improve. Which is greater: risk or benefit? What is in the best interest of the infertile couple?

References


Declaration: SL is CEO of Lewis Fertility Testing Ltd, a spin out company from Queens University, Belfast.

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